

NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE ON AGING
INTRAMURAL RESEARCH PROGRAM



**Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study
Wave 3**

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Lay Summary

The Healthy Aging in Neighborhoods of Diversity across the Life Span study (HANDLS) is a multidisciplinary, community-based, prospective longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities among socioeconomically diverse African Americans and whites in Baltimore. This study investigates whether health disparities develop or persist due to differences in SES, differences in race, or their interaction. This study is unique because it will assess over a 20-year period physical parameters as well as evaluate genetic, biologic, demographic, psychosocial, and psychophysiological parameters of African American and white participants in higher and lower SES. It also employs novel research tools, mobile medical research vehicles, in hopes of improving participation rates and retention among non-traditional research participants. The domains of the HANDLS study include: nutrition, cognition, biologic biomarkers, body composition and bone quality, psychophysiology, physical function and performance, sociodemographics, psychosocial, neighborhood environment and cardiovascular disease. Utilizing data from these study domains will facilitate understanding the driving factors behind persistent black-white health disparities in overall longevity, cardiovascular disease, and cognitive decline.

HANDLS Wave 1, the initial recruitment and examination phase that began in August 2004, is complete. The current protocol outlines the revisit waves of this study HANDLS Wave, 2 entitled “The Association of Personality and Socioeconomic status with Health Status – An Interim Follow-up Study” which began in June 2006 under a separate protocol is designed as a telephone interview to occur approximately 18 months after the first visit to the mobile Medical Research Vehicles (MRVs). Wave 2 provides interim contact with study participants, and important interim information regarding their health. The next visit HANDLS Wave 3 will consist of the second visit to the mobile Medical Research Vehicles (MRVs) for a health examination to occur at least 3 years after the first, a telephone interview to include the dietary interview and questionnaires, and for a random sample of those who participate in the revisit a direct measurement of renal function and a brain scan study using MRI to examine the structure of the brain.

Objectives and Aims

The primary objective of HANDLS is to conduct a longitudinal study of minority health focused on investigating the differential influences of race and socioeconomic status on health in an urban population.

The scientific research questions for this multidisciplinary epidemiologic study of minority health and health disparities are:

- Do race and SES influence health disparities independently or do they interact with several factors (race, environmental or biologic factors, and cultural or lifestyle practices)?
- What is the influence of SES and race on age-related declines in function in an urban population?
- What is the influence of SES and race on the incidence and natural history of age-related disease?

- Are there early biomarkers of age-related health disparities that may enhance our ability to prevent or ameliorate the severity of these diseases?

For specific systems we will test the following hypotheses during Wave 3 of HANDLS:

Cardiovascular – There will be significantly greater decline in cardiovascular health status as a function of SES and race independent of the effects of age in both men and women. For example: Left ventricular mass, an important cardiac risk factor, is greater in African Americans than whites and is greater in African Americans of lower SES as compared to age-matched African Americans with higher SES, in both men and women.

Body Composition and Bone Quality – Compared to white adults of comparable age, African Americans have:

- A higher proportion of fat to lean mass of the total body, trunk and extremities, and greater odds of meeting DXA-defined criteria for sarcopenia and sarcopenic obesity
- Lower bone density and greater odds of osteopenia and osteoporosis relative to their body weight

Compared to white adults of comparable age, African Americans will exhibit:

- Faster loss of lean mass, greater accumulation of fat mass and greater increase in the proportion of fat to lean mass of the total body, trunk and extremities, and greater risk of transition to sarcopenia and sarcopenic obesity
- Faster decline in bone density, and greater risk of transition to osteopenia and osteoporosis.

The above associations are correlated with, if not mediated by differences in health habits (nutrition, physical activity)

Cognition – The rates of decline of various cognitive abilities will be the same in all groups regardless of race, ethnicity, or SES.

Muscle Strength

- African Americans will exhibit better preservation of muscle strength into older ages than other ethnic and racial groups;
- African Americans have the same trajectory of muscle loss as other ethnic or racial groups after accounting for differences in occupational history, nutrition, and body mass and composition;
- All ethnic and racial groups will show the same relationships among changes in muscle strength, physical activity, and cardiovascular fitness regardless of socioeconomic factors, nutrition, and comorbid conditions such as diabetes.
- The greater strength reductions at older ages among lower SES individuals will be attributable to their greater severity of chronic diseases.

Covariates – Other variables such as nutrition, environment and neighborhood effects, genetic make-up, family history, activity level, access to health care, and prevalent medical, dental, psychiatric conditions, caregiving status, renal function oxidative stress, and DNA repair capacity may modulate

the effects of SES and race on cardiovascular, musculoskeletal, and cognitive functioning. For example:

- Nutritional Domain will examine the effects of race socioeconomic status (SES) on nutritional status and identify nutritional factors that may contribute to health disparity in cardiovascular and cerebrovascular health and cognitive function.
- Oxidative Stress/Inflammatory State Domain: The early appearance and increased severity of age-associated disease among African Americans and low SES individuals suggests that the factors contributing to the emergence of health disparities may also induce a phenotype of 'accelerated aging'. We seek to understand the underlying biologic, genetic, and environmental factors that may result in this phenotype. We hypothesize that in low SES populations and minority populations with high rates of early onset age-associated disease the interaction of biologic, psychosocial, socioeconomic and environmental factors may result in a phenotype of premature aging or accelerated aging biologically similar to heritable 'progeroid' syndromes whose manifestations include increased susceptibility to oxidative stress, premature accumulation of oxidative DNA damage, defects in DNA repair and higher levels of biomarkers of oxidative stress and inflammation. While genetic background, environmental and behavior factors influence health outcomes in all populations over the lifespan, health disparities may be the end product of accelerated, dysfunctional interactions of these factors in populations at high risk. HANDLS is examining this hypothesis by measuring biomarkers of oxidative stress and inflammation, assessing levels of the most widely studied oxidative DNA adducts, and measuring DNA repair capacity (DRC) in study participants. In addition, other important biomarkers of oxidative stress are being evaluated. These include glutathione levels, fluorescent heme degradation products, and plasma carbonyl levels. Measures of inflammatory states include the pro-inflammatory cytokines such as IL-17, MCP-1, IL-23, TNF- α , GRO-1, C-reactive protein, and uric acid. Prospectively measuring biomarkers of oxidative stress in a longitudinal study may clarify whether oxidative stress plays a pivotal role in aging and in the development and or progression of age associated disease. It may also provide insights into the different trajectories of aging observed in individuals.
- Genomic Domain:
 - Genetics: Current technological advances in genotyping now permit high throughput whole genome SNP genotyping to proceed with the overall goal of examining the genetic contributions to the development of multi-gene complex clinical disorders. Of equal importance is the contribution this new knowledge will provide in furthering the examination of the genetics behind the differences in medicinal drug responses frequently seen in individuals as well as to the discovery of new drug targets for a range of diseases with persistently high morbidity and mortality. HapMap and the continuing advances in genotyping technologies provides an important pathway to facilitate more directed (and more fruitful) candidate-gene, linkage-based and genome-wide association studies of commonly occurring complex diseases. However, to fully actualize its potential, it is important to address the gaps in our knowledge that still exist with respect to minority health and health disparities. To identify the genetic factors that are associated with age-associated health disparities we have already begun whole genome SNP genotyping using the Illumina Infinium II platform. Analysis of the first 1000 participants will be completed by winter 2009. Planned analyses will include analysis of admixture that will be done in collaboration

with Andrew Singleton, Chief of the Laboratory of Neurogenetics. Analysis of the data set is also underway to determine genetic associations with hypertension and other age associated health disparities. We will also investigate the frequency of genetic polymorphisms in DNA repair genes involved base excision repair specifically XRCC1 and OGG1.

- **Epigenetics:** The disproportionate incidence and mortality from age-associated disease may also result from epigenetic mechanisms such as DNA methylation. One theory of aging focuses on the role of genes and the epigenome in the development of the aging phenotype. We will examine the hypothesis that human disease and disability may result from DNA modifications that are not the result of a change in the coding sequence of genes. The clinical relevance of DNA methylation states in the development of age-related disease has yet to be understood on a population basis. There is variation in methylation states from individual to individual. This may be related to age, gender, environmental exposure, and other genetic factors. Is it possible that our hypothesized phenotype of accelerated aging phenotype seen in low SES and minority communities is related to epigenetic factors such as methylation? We will examine methylation states within this longitudinal cohort to attempt to understand whether methylation states are associated with the premature development of age-associated disease. Because there is limited information about methylation status of lymphoid cells, we have chosen to employ DNA isolated from the buccal cells for this study. This is also likely the best source of DNA in our urban based cohort at higher risk for environmental exposures from air pollution and because of the prevalence of tobacco and alcohol use within this cohort at higher risk for the development of aerodigestive cancers of the lung and esophagus. Our investigations will focus on identifying DNA methylation patterns factors that are associated with the development of health disparities and with changes in human DNA repair capacity. These studies will examine the gene promoter methylation status in buccal mucosa cell DNA from HANDLS participants. Assessing this at baseline and longitudinally may permit us to identify molecular markers of disease susceptibility especially for aerodigestive malignancies that are characterized by disproportionate incidence and mortality rates in African Americans.
- **Renal Function Domain:** African Americans have a 4-fold increased risk of developing end stage renal disease (ESRD) when compared to white Americans.¹ Risk factors that account for this increased susceptibility include disproportionate rates of hypertension, diabetes mellitus, as well as selected other conditions including systemic lupus erythematosus.² However, more recent studies also suggest that significant risk is imparted by life course socioeconomic status and neighborhood conditions.³ Identifying those at risk for the early stages of chronic kidney disease (CKD) is critically important in reducing the incidence of ESRD.

In clinical practice, CKD is most commonly detected by the presence of an estimated glomerular filtration rate (eGFR) $<60 \text{ mL/min/1.73m}^2$ with or without albuminuria, or $>60 \text{ mL/min/1.73m}^2$ with urine albuminuria.⁴ The eGFR is generally calculated using the Modification of Diet in Renal Disease 4-variable equation, which includes serum creatinine.⁵ However, several recent studies suggest that this may not be the most accurate means of estimating degree of kidney disease.

In hopes of providing early identification of participants with CKD, to improve outcomes and awareness of CKD among participants, serum Cystatin C levels and urinary kidney injury molecule-1 (KIM-1) will be measured in each participant. Cystatin C has been selected because the literature suggests that it may provide a more accurate estimate of GFR, especially when GFR is only mildly depressed.⁶ Additionally, Cystatin C has been found to be a better predictor of cardiovascular mortality than creatinine among persons with mild CKD.⁷ Urinary KIM-1 has recently been shown to be increased in patients with non-diabetic CKD and may be an important target for treating CKD.⁸

In contrast to the higher rate of ESRD, studies in representative populations show lower prevalence of earlier stages of CKD in African Americans compared to Whites when determined by creatinine-based eGFR.^{9,10} However, creatinine is affected by muscle mass, which varies by race and ethnicity, and therefore this discrepancy may reflect either hyperfiltration (i.e. true higher levels of GFR) or inaccuracy of the estimating equations in African Americans.^{11,12} In addition, the GFR estimating equations that have been used to determine these prevalence estimates have been developed primarily in populations with CKD, and are therefore limited in their ability to provide the most accurate information about kidney function in populations without chronic kidney disease. Therefore the largest gaps in our knowledge are: What are usual levels of GFR in racial and ethnic groups? Are filtration markers other than creatinine affected by race and ethnicity, independent of GFR?

In collaboration with Dr. Josef Coresh and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), a research group formed to develop and validate improved GFR estimating equations for glomerular filtration rate, we plan to implement a direct measurement of GFR using plasma clearance of Iohexol. Data from HANDLS participants will be pooled with data from participants in the Multi-ethnic Study of Atherosclerosis (MESA) at Columbia University, the University of California at Los Angeles, and Johns Hopkins University to provide actual measurements of GFR determine normative values for GFR in a multi-ethnic sample by age, and for use in development of accurate equations for estimating GFR in a broad range of populations. The aims for this domain include:

- i. Compare measured GFR across ethnicities in a population based sample of young individuals
 - ii. Relate measured GFR to known HANDLS covariates (SES, albuminuria, CKD risk factors)
 - iii. Relate new genetic susceptibility loci to measured GFR and kidney phenotypes in HANDLS
 - iv. Store specimen for measurement of established & novel GFR markers. This will contribute to an equation for estimating GFR across age & ethnicity. Stored specimens can be used for other future studies.
 - v. Contribute data to development of equations for estimating GFR across age & ethnicity
- Caregiving – Health disparities may result from various forms of stress including psychological stress. Many studies have linked caregiving with significant levels of chronic stress for caregivers. This chronic stress is moderated by socioeconomic status, the condition and disabilities of the individual for whom care is provided, social support, and the age of the caregiver. While depression is a well studied health come among caregivers, other studies have shown that overall health, compliance with appropriate health related behaviors, and diet are all negatively influenced by caregiving. There are a few studies that have examined

the effects of accumulated multiple social roles (i.e. caregiver, spouse/partner, parent, and employment, and volunteer) and role combination (e.g. elder care, only; child care only; elder care and child care).¹³⁻¹⁸ This body of literature supports either the *scarcity hypothesis*, occupancy of more than one role is associated with poor well-being (e.g. Hong & Seltzer¹⁴); while others support the *enhancement hypothesis*, occupancy of more than one role is associated with positive outcomes (e.g. Adelman¹⁹). Most of this research included a primarily White sample of caregivers. There remains a lack of research focused on middle and older aged, African-American, women who are in multiple caregiving roles. To examine the influence of multiple caregiving roles (i.e., occupancy of more than one caregiving role) on the physical and mental health outcomes of HANDLS participants with specific focus on grandmother caregivers. This aim is to gain greater understanding about the relation between multiple caregiving roles (i.e., occupancy of more than one caregiving role), and health status (physical and mental) among HANDLS participants. This proposed study could extend the caregiving literature in several ways. First, it will assess the influence of multiple caregiving roles on health status of caregivers, across race/ethnicity, class and gender. Previous studies lacked sample diversity and primarily focused on low-income, African Americans, and/or grandmothers. Inclusion of a diverse sample will allow the researcher to examine intra and inter variations based on caregivers' age, race/ethnicity, sex and education. Second, it will assess the influence of role combination, (e.g. elder care, only; grandchild care only; elder care and grandchild care). Several researchers found that role combination may have a greater influence on health outcomes than simply the number of roles.¹⁴

- Neuroimaging domain – There are pronounced health disparities associated with race and socioeconomic status (SES) in various brain health endpoints including stroke, dementia, cognitive decline, and functional disability.^{20,21} Particularly potent race disparities in stroke incidence are apparent at strikingly young ages, with a four-fold increased risk of stroke mortality among 45-59 year old African Americans (AA).²² Efforts are needed at disentangling the respective influences of race and SES in brain health, particularly early and subtle markers of brain pathology that predict future stroke, dementia, or cognitive and functional decline. Measures of subclinical or covert cerebrovascular disease assessed by magnetic resonance imaging (MRI), including gray matter and white matter volumes and white matter microstructure, offer such proven associations.^{23,24} Identifying multi-level mediators of the relations of race and SES to subtle brain pathology is also crucial. Biomedical, behavioral, psychological, social, and environmental factors have been implicated as potential mediators of the relations of race and SES to a multitude of physical health outcomes,^{25,26} but little is known about these pathways for brain health endpoints.^{26,27} Recent quantitative MRI data in older adults revealed larger brain volumes, but greater white matter hyper-intensities in African Americans than whites.²⁸ The most pronounced relations of vascular disease to brain atrophy and white matter hyper-intensities were found in African Americans. MRI indices of subtle brain pathology have been associated with lower levels of cognitive and physical function and cognitive decline,^{29,30} and may mediate relations of race and SES to these endpoints.

This protocol is an ancillary project linked to the ongoing HANDLS study. In a subset of 500 HANDLS participants, we will assess total and regional gray matter and white matter volumes and white matter microstructure in 500 stroke- and dementia-free HANDLS participants (250 African American, 250 white; 50% women; ages 30-64 at baseline) over the full range of socioeconomic status using quantitative MRI data, including volumetrics and diffusion tensor imaging (DTI). We will address the following aims and hypotheses:

Specific Aim 1. Examine race- and SES-related health disparities in MRI-assessed measures predictive of future stroke, dementia, or cognitive decline, and evaluate whether these relations differ by sex and age. The primary outcome measures will include total and regional gray matter and white matter volumes quantified by voxel-based morphometry, ischemic lesion volumes, and total and regional fractional anisotropy (FA) and the apparent diffusion coefficient (ADC) estimated by DTI.

Hypothesis 1. There will be significant interactive relations of race and SES with respect to MRI indexes of gray matter and white matter volumes, ischemic lesion volumes, and white matter microstructure such that lower SES African Americans will display the most extensive brain pathology, particularly in prefrontal regions. Moderated mediation by age and sex (i.e., that age and sex may moderate the mediational paths by which race and SES relate to brain outcomes) will be explored

Specific Aim 2. Examine multi-level mediators of the relations of race and SES to brain MRI outcomes; potential mediators (i.e., vulnerability or resilience factors) include biomedical (e.g., cardiovascular risk factors, subclinical vascular disease, cardiovascular comorbidities), behavioral (e.g., diet, smoking, alcohol, physical activity), psychological (e.g., depression, vigilance, anger, stress, spirituality), social (e.g., social support and networks, racial discrimination), and environmental (e.g., neighborhood deprivation, access to health care) factors.

Hypothesis 2. The multi-level mediators of MRI-based measures of GM and WM will differ as a function of race and SES. For example, select psychological factors such as racial discrimination may be prominent influences in high SES African Americans (as per pilot data), whereas behavioral, social, and environmental factors may be the most prominent influences in low SES African Americans. Moderated mediation by age and sex will be explored.

Specific Aim 3. To examine whether MRI indexes of gray matter and white matter are proximal mediators of the relations of race and SES to cognitive and physical function.

Hypothesis 3. Lesser white matter integrity and lesser white matter and gray matter volumes, and higher ischemic lesion volumes will be associated with lower levels of cognitive (particularly executive) function and physical function. These associations will be most pronounced among lower SES African Americans. Moderated mediation by age and sex will be explored.

Background and Significance

There are well-documented differences in health status among groups defined by age, race, ethnicity, and SES. Over the past decade or so, evidence from cross-sectional studies and nationally representative follow-ups suggests that there are persistent disparities among African Americans and other minority groups compared to Whites in morbidity³¹⁻⁴⁶ and mortality.⁴⁷⁻⁵³ Double jeopardy describes the constellation of health disparities conferred by old age and membership in a minority group.⁵⁴ Evidence suggests that there are unique disadvantages conferred by the combination of old age and minority status,^{31-37,39,41-49,51,54-57} but the extent to which minority status is a direct cause of the disadvantage is unknown. Race, ethnicity, and SES are inextricably confounded in many studies. Membership in a minority group may be an indicator of the combinations of other effects such as

low income, poor education, environmental exposure to toxic compounds, and lack of occupational opportunities.

Independent of the effects of race and ethnicity, SES accounts for differences in the functional status associated with chronic disease, but has only a small role in predicting prevalence of chronic disease.⁴⁶ Further complicating this relationship, physicians' assessments and treatment differ by race and sex.^{55,58} Addressing these disparities in health status requires data about the differences in risks for chronic disease associated with race, ethnicity, and SES in all groups regardless of their majority or minority standing.

The scientific objectives of HANDLS are to establish a single-site prospective longitudinal epidemiologic study of health disparities in socioeconomically diverse African Americans and whites residing in the city of Baltimore. Specifically, we designed HANDLS to disentangle the effects of race and SES on risk factors for morbidity and mortality, to examine the incidence and progression of pre-clinical disease, and to follow-up the development and persistence of health disparities, longitudinal health status, and health risks. The mechanisms or biologic and molecular pathways through which the health and longevity trajectories of individuals in American society are influenced are unknown at this time.

The present proposal focuses on predictors of change in cardiovascular function and fitness, risks for cerebrovascular conditions such as stroke, vascular dementia, and carotid stenosis, renal function and pathological cognitive decline. We chose these specific areas as representing the health issues that are among the most prevalent, but least understood, in African Americans and low SES urban dwelling whites who have health burdens similar to African Americans. Specifically, we will measure carotid arterial blood flow and arterial stiffness by Doppler ultrasonography, muscle strength by grip strength, chair stand and single leg stand exercises, body composition by dual photon x-ray absorptiometry (DXA), glomerular filtration rate and cognitive performance with cognitive and neuropsychological tests, and neuroimaging parameters by MRI.

We assess each of these areas by separate procedures for which we will investigate cross-sectional differences and longitudinal change within this sample and by comparison with other samples, particularly the Baltimore Longitudinal Study of Aging (BLSA) and other studies with which this study shares many procedures and tests. We will combine these measures in various ways to examine the risks for pathological outcomes such as stroke, dementia, and loss of functional independence.

Study Design

The HANDLS study is a multidisciplinary, prospective epidemiologic longitudinal study examining the influences and interaction of race and SES on the development of cardiovascular and cerebrovascular health disparities among minority and lower SES subgroups.

The baseline HANDLS sample consists of 3724 community-dwelling African American and white adults aged 30-64. Participants were drawn from 12 pre-determined census tracts in Baltimore City, sampling representatively across a wide range of socioeconomic and income circumstances. The heuristic study design is a factorial cross of four factors: age, sex, race, and SES with approximately equal numbers of subjects per "cell." (Figure 1) HANDLS is planned as a 20-year longitudinal study of the 3724 individuals accrued (Figure 2). Using our mobile medical research vehicles, we will now re-visit each census tract for 2-3 months over the next 3 years.

The 12 census tracts identified were selected because they were likely to yield representative distributions of individuals between 30 and 64 years old who are African Americans and whites, men and women, and lower and higher SES.

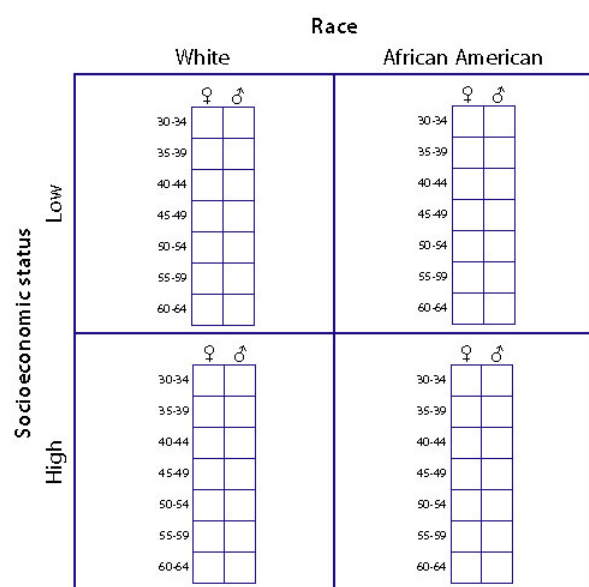


Figure 1. HANDLS sampling design

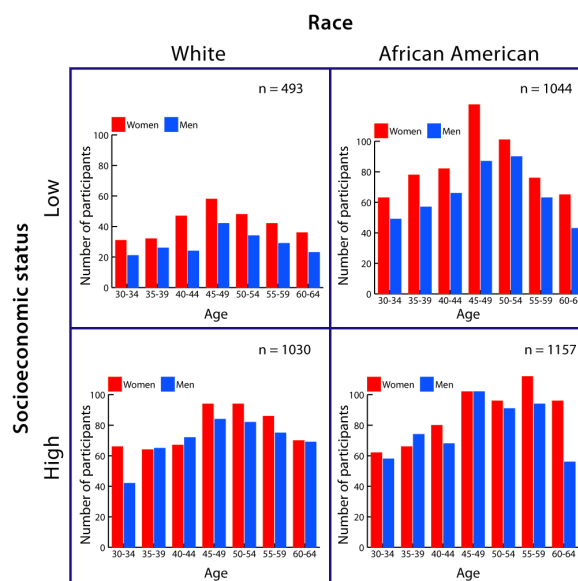


Figure 2. HANDLS baseline accrual

Study Sample

The study has recruited a representative sample of whites and African Americans between 30 and 64 years old from twelve census tracts in Baltimore City in both low and high socioeconomic strata as a fixed cohort following the overall design. By collecting a baseline assessment and 5 follow-up triennial assessments over approximately 20 years, there will be sufficient power (>.80) with 30 participants per group (race by SES by sex by age group) remaining after 20 years. There will also be sufficient power (>.80) to compare rates of change among groups after the baseline assessment.

Inclusion Criteria:

- Verified HANDLS participants
- Able to give informed consent
- Must have valid picture identification

Exclusion Criteria:

- Pregnancy
- Within 6 months of active treatment of Cancer (Chemotherapy, biologic, radiation)

Data Management

Data are kept in medical charts in locked file cabinets. All clinical research forms are filed in locked file cabinets. These materials are kept within a locked medical record room. Access to all study data is limited to HANDLS staff and investigators. Data are coded and entered by ID number only. Collaborators receive ID numbers only. No other identifying information is provided with the data.

Data Analysis

The study employs a standard statistic software package depending on the independent and dependent variables being analyzed. Data analyses include logistic regression and mixed effects modeling.

Facilities

Phase 1 participant visits occur in the field on the medical research vehicles. The vehicles have computer facilities for initial data entry. Computers located at the Gerontology Research Center, the NIH Biomedical Research Center and the ASTRA Unit at Harbor Hospital provides additional computer support for data analysis and clean-up. The Nichols Institute of Quest Diagnostics in Chantilly, Virginia will do the clinical laboratory work. DNA, serum, plasma, and saliva samples from consented participants will be prepared and stored in the NIA Clinical Core Laboratory. Research lab values are determined in CLIA approved intramural research program labs or sent out to private sector clinical laboratories.

Project Organization

There are two MRV physicians, a nurse practitioner, registered nurse, ultrasonographer, cardiovascular technician, approximately 4 or 5 psychological testers to administer the cognitive battery, community coordinator, two security guards, vehicle drivers, and 2 vehicle logisticians. All HANDLS personnel report to the principal investigators as most are assigned to the Clinical Research Branch of the NIA IRP. The security guards, while specially trained for fieldwork on the vehicles, report to the head of security at the NIA IRP who is a member of the HANDLS logistics team. The Director of Security reports to the principal administrator of the NIA IRP, and therefore to the Scientific Director and Deputy Scientific Director of the IRP. The principal investigators have been engaged in this research project for 11 years to date beginning with vehicle conception and design, support of the pre-pilot community-based research, development and implementation of the pilot, and ongoing development of the large planned population-based study.

Project Schedule

March 2009	Complete Baseline Evaluation
April – June 2009	Staff training MRV preparation for wave 3 study
July 2009 – Dec 2010	Conduct wave 3 field studies of census tracts in Baltimore City

Problems or Weaknesses

The weaknesses and potential problems of this study are similar to other longitudinal epidemiological studies, re-contact and retention of participants. Thus far our Interim re-contact study which reaches out to participants approximately 18 months after initial baseline visit completion has been able to re-contact approximately 74% of participants initially seen between 2004 and 2006. This hopefully will lay the groundwork for the beginning of the next examination wave that will commence in spring 2009.

Methods

The study data for wave 3 will be collected in three parts. We will collect the first part of the participant examination data on the medical research vehicles. These data include an interim medical history and physical examination since the baseline examination; dietary recall; cognitive evaluation; echocardiography; assessments of muscle strength and bone density; laboratory measurements (blood chemistries, hematology, biomaterials for genetic studies); and, an audio-administered questionnaire. We will collect the second part as a telephone survey. It includes a repeated dietary recall interview and a caregiving assessment instrument. A portion of the participants will be invited to participate in the third parts of wave 3, the GFR ancillary study and the neuroimaging ancillary study. We will conduct the GFR study at Harbor Hospital's Astra Unit. We will conduct the neuroimaging study at University of Maryland School of Medicine.

PHASE 1: Medical Research Vehicle Examination Phase

Measure or Procedure	Estimated Timing	Location
Consent	20 minutes	MRV 3
Specimen Collection (Urine, Blood, Buccal Smear)	20 minutes	MRV 3
Anthropometrics	5 minutes	MRV 1
Interim Medical History	20 minutes	MRV 1
Interim Physical Exam	20 minutes	MRV 1
Dietary Recall I	30 minutes	MRV 2
Cognition	40 minutes	MRV 2
Physical Performance	15 minutes	MRV 1
Echocardiogram	20 minutes	MRV 1
Questionnaires Section A	35 minutes	MRV 2
Body Composition/Bone Densitometry	30 minutes	MRV 1

The following procedures will be implemented on the MRVs after obtaining informed consent for the first phase of the participant examination:

Medical history and physical examination. A physician or nurse practitioner will perform an interim physical examination and medical history. The purpose of the physical examination and medical history is to document as unambiguously as possible any diagnosable conditions, to record medications and their frequencies and dosages, and to assess disabilities that might limit independent

functional activities, that have developed or occurred since their last examination on the MRVs. In addition, we will examine subjects to insure that they do not meet exclusionary criteria for any subsequent tests such as the DEXA.

Risks. None are known.

Fasting blood samples for List Tests, Banking Plasma, Serum, and DNA. As a part of the medical evaluation, blood tests are performed to look for anemia and other blood disorders, diabetes mellitus, thyroid disease, hepatitis, prostate disease, HIV disease and kidney disease. We are also using some blood samples to study genes that may play a role in age-related diseases like Alzheimer's disease, heart failure, high blood pressure, and cancer. The total amount of blood drawn from each participant is about 62 milliliters (4½ tablespoons).

Risks. There are some risks from having blood drawn. There is a risk of an infection from the needle puncture. There is also a risk of a black and blue mark, and the participant may feel faint. It is common to have a small black and blue mark, but it disappears after a day or so. Some people may begin to perspire or feel nauseated. These risks are very small. Our medical staff is well trained and has drawn blood many times.

Buccal Cell Collection: As part of the medical evaluation Buccal mucosa cells will be collected from saliva samples using the DNA Genotek Oragene RNA and DNA self collection kit from each consenting participant. Participants will be asked to spit into a DNA collection system and a RNA collection system (a small sample cup) to collect buccal mucosal cells. The extracted DNA and RNA will be used for epigenetic analysis as well as human mRNA expression profiling.

Risks. This is a completely non-invasive self-collection system. There are no known physical risks.

Dietary Recall. This measure will be administered in both the first and second phases of data collection. We will ask participants to recall all of the foods and beverages they consumed during the previous 24 hours. An interviewer will record the dietary recall using methods developed by the USDA called the Automated Multiple Pass Method that is supplemented by measurement aids and illustrations to assist in estimating accurate quantities consumed.

Risks. None are known.

Cognitive testing. We will administer a battery of cognitive tests assessing memory, executive function, verbal fluency and knowledge, and spatial ability. In addition to dementia screening using the Mini-Mental State Examination⁵⁹, we will administer the Benton Visual Retention Test (BVRT),⁶⁰ California Verbal Learning Test,⁶¹ Card Rotations, Prospective Memory, Wechsler Adult Intelligence Scale Digit Span Forward and Backward,⁶² Identical Pictures, Clock Drawing, Brief Test of Attention, Wide Range Achievement Test, Trail Making A and B, animal fluency. We will assess baseline personality and symptoms of depression using the CES-D.

Risks. None are known.

Audio-administered Questionnaires. We will assess risk of poor mental health and questions about food security and income with an audio-administered (using a computer and headphones) questionnaire. Assistance will be provided to the participants, if for example they have trouble seeing or reading the questions or are uncomfortable with using a computer.

Risks. None are known.

Echocardiogram. Echocardiography is an ultrasound test that is the preferred exam for the non-invasive assessment of the structure and function of the heart. We will measure the dimensions of the chambers of the heart, the thickness of the walls, and the systolic and diastolic function of the chambers. We will also examine the structure and function of the valves. This test does not involve radiation, there are no known risks (except for irritation from electrodes), and there are no exclusions.

Risks. None are known

Bone Density and Body Composition. We will perform dual energy X-ray absorptiometry (DXA) on total body, lumbar spine, the hip and the Instant Vertebral Assessment (IVA) using a Discovery QDR series (Hologic, Bedford MA). DEXA delivers a small amount of radiation through an X-ray source while you lay on the scanner bed. Site-specific scans of the lumbar spine and right hip provide information on bone area (cm²), and bone mineral density (g/cm²). Total body scan measures both body composition and bone mineral density, including bone mineral content (g), bone area (cm²), bone mineral density (g/cm²), total body tissue (g), fat mass (g), lean mass (g), lean mass plus bone mineral content (g), and percent total fat (%). The IVA provides an assessment of vertebral fractures. Results of the total body scan are presented for the body as a whole as well as for the arms, legs, trunk, head, pelvis, and spine.

Exclusions. DXA studies are not administered to pregnant women or individuals who have had both hips replaced. Individuals weighing greater than 450 pounds are excluded due to the densitometer's limitations.

Risks. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study and has approved this use as involving minimal risk and necessary to obtain the research information desired. Although each organ will receive a different dose, the amount of radiation exposure participants will receive from these procedures is equal to a uniform whole-body exposure of less than 1 millirem. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. The amount of radiation received in this study is within the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is an effective dose of 5 rem (or 5,000 mrem) received per year.

Table 1. Radiation associated with DEXA studies on spine, femur, vertebrae and whole body.

Scans	Millirems
Anterior-posterior spine, DXA	0.7
Anterior-posterior femur, DXA	0.7
Lateral Scan for IVA	7.0
Total body scan, DXA	1.0

The NIH Radiation Safety Branch monitors equipment and technique used in this study.

Age-associated strength loss (Grip Strength Test). Handgrip strength in both hands, measured using an adjustable, hand-held, hydraulic grip strength dynamometer, will be used as an overall assessment of physical strength and skeletal muscle function. Repeated measurement of grip strength over the follow-up visits will permit an estimate of strength loss over time. Grip strength is a commonly used

indicator of health status and physical frailty and mid-life grip strength has been shown to be a strong predictor of early mortality.

The examination is done with the participant in the sitting position with the arm to be tested resting on the table and the elbow held at approximately a right angle. The dynamometer is held in the hand to be tested and is resting on a mouse pad. The participant is instructed to grip the two bars of the dynamometer in their hand, and to slowly squeeze the bars as hard as they can. The test is repeated on the other hand. This test is performed 3 times on each hand.

Exclusions. Participants who have had fusion, arthroplasty, tendon repair, synovectomy, or other related surgery of the upper extremity in the past 3 months will not be tested on the affected hand.

Risks. None are known.

Age-associated functional decline.

Sit-to-Stand Test. A commonly used performance-based test of physical function, the sit-to-stand test (also termed repeated chair stands), will be used to assess functional status at study inception and to track loss of functional capacity over time. Using a standard armless chair placed securely against a wall, the participant is first instructed to rise from the chair without using arms and return to a seated position. If this is done successfully, the participant is then asked to repeat that movement 10 times. Performance, both whether 10 stands are completed and time to perform 5 or 10 stands has been strongly associated with onset of functional limitation, physical disability, institutionalization, and mortality.

Exclusions. There are no formal exclusions from attempting the single chair stand; inability to rise from a chair without using arms excludes participants from doing repeated chair stands.

Single Leg Stand Test. The single leg stand test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur. This test requires the participant to stand on one leg with the other leg flexed at the knee and held about two inches from the floor. The participant is asked to hold the position for as long as they can, up to 30 seconds. The single leg stand has been found to be a sensitive test of standing balance for middle age and older adults and has been used in numerous epidemiologic studies of well elderly without mishap.^{63,64}

Phase 2 – Post-examination Telephone Survey

Measure or Procedure	Estimated Timing	Location
Dietary Recall II & Supplement Questionnaire	30 minutes	Telephone
Questionnaire (Income/Wealth Assessment)	15 minutes	Telephone
Care-giving Questionnaire	15 minutes	Telephone

Dietary Recall. We will ask participants to recall all of the foods and beverages they consumed during the previous 24 hours during a telephone interview. An interviewer will record the dietary

recall using methods developed by the USDA called the Automated Multiple Pass Method that is supplemented by measurement illustrations to assist in estimating accurate quantities consumed.

Nutrition Supplement Questionnaire. We will ask participants to report all of the types and quantities of nutritional supplements they took during the previous 24 hours following the dietary recall. An interviewer will also record usual supplement practices.

Risk/Benefit Assessment

There is very little risk to participants in this observational study. The exposure to low dose radiation from the analysis of bone density and body composition by the densitometer and the risks associated with having blood drawn are the minimal risks. The HANDLS GFR measurement protocol carries slightly more risk that we will mitigate by excluding those with conditions that have been associated with side effects from the contrast material.

The potential benefits to the participants include access to a full medical evaluation including screening for pathology in which early detection is advantageous. If the study doctor discovers any condition or problem, the information will be provided to the participant immediately and their primary care doctor, with their permission. If the participant does not have a physician, efforts will be made to refer them for care. Participants will be reimbursed for time and inconvenience.

The potential benefits to society relate to improvement of overall health in a vulnerable population that currently bears a disproportionate burden of disease and disability in this country. Healthy People 2010, the nation's disease prevention agenda, have defined two national goals to reduce preventable threats to the nation's health.⁶⁵ The first is to increase the quality and years of healthy life and the second is to eliminate health disparities. However, in order to achieve this second goal it is critical to develop research initiatives that provide new insights into the relationship between psychosocial factors and health status by (1) incorporating biological measures into large scale epidemiologic health and survey research projects and (2) the development and inclusion of a diverse panel of biomarkers or biologic measures that evaluate biologic pathways that may be involved in the causal relationship between SES and health.⁶⁶ This is what HANDLS attempts to accomplish. If successful, HANDLS will provide unique information that will hopefully uncover findings that will provide a basis for the development of appropriate prevention and intervention strategies to reduce health disparities.

Compensation

Participants may be paid up to \$220 for participating in phase 1 (\$160) and 2 (\$60) of this study. If they participate in phase 3A (GFR study) they will be compensated an additional \$100. If they participate in phase 3B (DTI study) they will be compensated an additional \$50.00. If a participant is unable to complete all of the tests they may receive a portion of that payment. They will receive payment in the form of an ATM debit card at the end of the MRV visit. The ATM card will be activated before they leave the vehicle. The participant will be instructed to take the card to an ATM machine in their neighborhood to withdraw payment.

Written instructions regarding how to access payment will be provided.

Ancillary study -- GFR Measurement Using Plasma Clearance of Iohexol

(1) Physical Description and Pharmacokinetics

Iohexol is a non-ionic contrast medium of low osmolality that was developed as a cheaper and safer alternative to the ionic contrast markers first used in imaging techniques. Introduced for use in radiographic imaging techniques in 1982, iohexol was first used as a GFR marker in Sweden in 1984 and has been extensively employed as both an imaging marker and an exogenous filtration marker in both clinical practice and research for the past two decades.

Iohexol has a molecular weight of 821.14 and iodine content of 46.36%. Commonly referred to by its trade name, Omnipaque, iohexol is available in multiple concentrations, the most commonly used for GFR measurement (Omnipaque 300) having 300 mg organic iodine/mL of solution. Once administered, iohexol is eliminated from the body by renal excretion with approximately 88% (73.1-98.2%) of the injected dose filtered out of the body through the kidneys in the first 24 hours in people with normal kidney function. No significant metabolism, deiodination or biotransformation occurs.

Plasma clearance of iohexol has been shown to approximate urinary clearance of inulin. In one study the regression slope and intercept were 0.95 and 5 ml/min/1.73 m², respectively, with a correlation coefficient of 0.97⁶⁷. In the single study that has compared ¹²⁵I-iothalamate and iohexol, the correlation coefficient and standard error of the estimate was 0.93 and 11 ml/min/1.73 m².⁶⁸

(2) GFR Measurement Protocol

A. Subject Selection

1. Inclusion criteria

All study participants aged 34-60 who are able to provide informed consent.

2. Exclusion criteria

Prior reaction to administration of contrast media; allergy to iodine or iodine-containing substances; current treatment for thyroid carcinoma; acute exacerbation of asthma or chronic obstructive lung disease in the past three months requiring hospitalization or oral steroid therapy; currently undergoing or having received either peritoneal dialysis or hemodialysis treatment within the past three months; solid organ, bone marrow or stem cell transplant; condition causing inability to have insertion of two intravenous catheters; cognitive or physical impairments that will not allow completion of the study.

B. Recruitment plan. We plan to recruit 300 participants 150 white and 150 African American participants as a random sample of participants returning for the wave 3 revisit wave. The recruitment process may include a face-to-face inquiry by the recruitment coordinator, a brochure describing the clinical problems associated with chronic kidney disease and the procedures, time for the recruitment coordinator to answer participants' questions, review of eligibility and exclusions, and signing the consent forms.

C. Power calculations. We estimated the mean estimated GFR (eGFR) for African Americans and Whites in NHANES III for the participants the same age as the HANDLS participants (34 to 60 years of age). Estimated GFR was calculated using both the Modification of Diet in Renal Disease (MDRD)

study equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) group equation (smaller coefficient for African Americans).

Linear regression was used to predict eGFR adjusting for age, sex and race. The coefficient for African Americans was used as the mean difference and the RMSE was used as the standard deviation.

MDRD study equation

Root MSE	25.09					
egfrcr	Coef.	Std. ERR	T	t P> t	[95% Conf. Interval]	
Black	11.04209	0.816914	13.52	0	9.440473	12.64371
Sex	-1.62753	0.805804	-2.02	0.043	-3.20736	-0.04769
Age	-0.63793	0.055789	-11.43	0	-0.74731	-0.52855
Intercept	110.9666	3.236187	34.29	0	104.6219	117.3114

CKD-EPI equation

Root MSE	15.762					
egfrcr	Coef.	Std. ERR	T	t P> t	[95% Conf. Interval]	
Black	8.281029	0.513185	16.14	0	7.274894	9.287165
Sex	0.771686	0.506206	1.52	0.127	-0.22077	1.764139
Age	-0.89861	0.035047	-25.64	0	-0.96732	-0.8299
Intercept	128.5786	2.032972	63.25	0	124.5928	132.5644

For a significance level of 0.05 and power of 0.8, we would require 82 people per group to detect a difference of 11 ml/min per 1.73 m² and 56 people per group to detect a difference of 8 ml/min per 1.73 m². Therefore, there is sufficient power to detect a mean difference of at least 8 ml/min per 1.73 m² between group for the projected sample size of 300.

With this sample size, the minimum detectable difference between groups would be 5 ml/min per 1.73 m assuming a standard deviation of 15 at this power and significance level, which is consistent with a clinically meaningful difference. Improved precision in the GFR measurement is expected to allow for greater power for detection of differences.

C. Prior to arrival. For those who agree to participate, a detailed set of instructions will be provided. Subjects will be asked to have a light meal the evening before and a light breakfast at home before the morning of the visit. Medications will be reviewed with the participant. They will be asked to avoid changes in medications that influence GFR (e.g. anti-inflammatory agents, diuretics, renin angiotension blocking agents). Patients on medications that interfere with creatinine secretion (e.g. cimetidine, trimethoprim) will be asked to stop the medications one week prior or will be excluded. All subjects will be asked to drink two to three glasses of non-alcoholic, non-caffeinated beverages prior to arrival for the study visit.

Measure or Procedure	Estimated Timing	Location
GFR	5 hours	Astra
DIS	75 minutes (concurrent)	Astra
Health Literacy (Rapid estimate of Adult Literacy in Medicine and Test of Functional Health Literacy in Adults)	15 minutes (concurrent)	Astra
National Endowment for Financial Education High School Financial Planning Program Session 1	60 minutes (concurrent)	Astra

D. Study day. A saline lock and intravenous line will be inserted at two different sites. A baseline blood sample will be taken and then 5 ml of Omnipaque 300 iohexol will be injected into one of the intravenous lines over a 60 second period followed by 10 ml normal saline flush. The syringe will be weighed before and after injection. Following injection, the intravenous line used for injection will be removed. Blood samples for plasma clearance measurements will be taken at approximately 10, 30, 120, 240 minutes from the second intravenous line. Times are approximate but the exact time will be recorded. Participants will be fed a low protein lunch consisting of approximately 15 to 25 grams. Participants are free to move around during the GFR test. After the final blood sample, the second intravenous line will be removed; participants will be observed for approximately 30 minutes and will return home. For participants with eGFR <30, a late blood sample at 360 minutes will be obtained.

A total of 4 mL of plasma is required at each time point, for a total of 24 mLs (5 draws plus 1 ml discard) for the entire visit. In addition, at baseline we will draw blood for creatinine, cystatin, albumin, hemoglobin, C-reactive protein and thyroid stimulating hormone. Samples will be collected in 4 mL green top heparin plasma tubes and baseline serum samples will be collected in a 10 mL red top tube. All plasma specimens will be centrifuged within 24 hours at 2000 G for 10 minutes, plasma will be removed and stored at -70°C. Specimens will be shipped on dry ice to University of Minnesota in batches.

E. Materials. Five milliliters of iohexol [Omnipaque 300 (300 mg organic iodine/ml of Iohexol); Nycomed Anasham] will be administered by a pre-weighted syringe beginning at time zero over a period of 1 to 2 minutes. Immediately post-injection, the syringe will be weighted to the nearest tenth gram on the same scale. The total administered dose will be calculated as follows:

$$\frac{[(\text{initial syringe weight} - \text{final syringe weight}) \times (\text{iohexol concentration})]}{1.345}$$

1.345

where 1.345 is the density of iohexol at room temperature.

F. Assay. Concentrations of iohexol will be determined using high performance liquid chromatography at University of Minnesota. The calibration curve consists of 6 standards that are assayed at the beginning of every batch to calibrate the assay. All specimens and two levels of QC are

assayed from each calibration. Every 5-10 specimens and at the end of the batch a standard is assayed and must read within 5% of the theoretical value to check that the assay remains in calibration. Aliquots of the filtrates, along with standards and controls, are injected into a high performance liquid chromatograph (Shimadzu SPD-10AV VIS/UV variable wavelength detector). Iohexol is chromatographed on a Supelcosil LC-18-DB column conditioned with an ion pair reagent. It chromatographs with two peaks. Detection is 254 nm. Quantification is achieved by measuring the peak height of the second iohexol peak relative to the standard peak height. The inter-assay CV is 2.99% at 10.0 mg/dL and up to 4.47% at 40 mg/dL; the intra-assay CV is <2.0%.

Risk/benefit Assessment

a. Physical Risk. All iodinated contrast materials have a risk of adverse reactions. The risk appears to be related to dose, osmolality, charge, and level of kidney function. At doses used for imaging procedures (50-200 ml), the incidence of severe adverse reactions is approximately 1-2 per 10, 000 administrations.

Adverse reactions associated with iohexol administration are usually mild to moderate in severity; however, on rare occasions, serious, life-threatening or fatal reaction, mostly of cardiovascular origin, have been associated with the administration of iodine-containing contrast media or dye. Mild reactions generally occur immediately or within 20 minutes of administration and are transient in nature. Commonly reported reactions are a transient feeling of warmth immediately following intravascular injection of iohexol. Mild symptoms that may be experienced include a sensation pain during injection, dizziness, lightheadedness, headaches, abnormal vision, nausea, diarrhea, or cramps. Other reported symptoms include anxiety, fever, being unable to move and speak, and convulsion.

One of the major concerns with the use of iohexol for imaging studies in patients with kidney disease is acute reduction in kidney function (contrast nephropathy), especially in patients with reduced kidney function. When using contrast for routine imaging procedures, the reported incidence of contrast nephropathy varied greatly depending on risk factors. The risk is negligible in patients with normal kidney function, even in patients with diabetes; 4 to 11% with moderate reductions in kidney function (serum creatinine 1.5 to 4 mg/dl); 9 to 38% in patients with moderate reductions in kidney function and diabetes, and 50% in people with serum creatinine >4 mg/dl (which is equivalent to kidney failure in most patients)^{69,70}. In one study of patients undergoing coronary angiography for coronary disease, an increase in plasma creatinine of more than 0.5 mg/dL above baseline, occurred in 3.3 percent overall and in 25 percent of patients with a baseline serum creatinine above 2.0 mg/dL. Most episodes are transient in nature and permanent kidney damage is rare.⁷¹

The manufacturer of iohexol, Amersham Health Inc., lists the relative occurrence of adverse reactions in clinical trials involving intravascular administration of iohexol in 1485 subjects on their package insert. The incidence rate of various adverse events was reported as follows: arrhythmias (2%), angina/chest pain (1%), hypotension (0.7%), other cardiac events (0.3% or less), vertigo (0.5%), pain (3%), vision abnormalities (2%), headache (2%), taste perversion (1%), respiratory reactions (i.e. rhinitis, cough, laryngitis) (0.2% or less), nausea (2%), vomiting (0.7%), urticaria (0.3%), purpura (0.1%), abscess (0.1%), and pruritus (0.1%). An analysis of the Sicilian Regional Centre for the Spontaneous Reporting of Adverse Drug Other studies report rates of adverse events of all categories in 0.09⁷² to 3%⁷³ of administrations, with severe events being reported in only 0.04%⁷³ to 0.004%⁷¹ of administrations. Appendix B, tables 1-3 list the adverse reactions and treatments by organ system, as compiled by the American College of Radiology (ACR) for the 2004 Manual on Contrast Media, 5th edition.

Risk factors for adverse reactions include history of asthma or bronchospasm, history of allergy or atopy, volume depletion, renal disease⁷¹.

The dose of 5 mL of Omnipaque 300 (1.5 g organic iodine) administered for GFR determination is 1/10th to 1/40th of the dose recommended for many body CT contains a total amount of iodine that is less than 2% of the maximum recommended dose of iodine. At doses used for GFR measurements, no reports of acute renal failure have been reported.^{69,74} A Swedish study by Nilsson-Ehle et al reported that of approximately 8000 iohexol GFR determinations in patients with and without reduced kidney function using a intravenous dose of 5 ml of iohexol, only 2 patients complained of any symptoms. The symptoms reported were transient malaise and vomiting between 1 and 3 hours after injection and it was uncertain whether this was indeed caused by the iohexol.⁷⁵

Given that many of the symptoms of reactions to iohexol appear to be anaphylactoid in nature, subjects with a history of reaction to contrast media or known allergies to iodine and/or iodine-containing substances are at a higher risk of experiencing adverse effects from the administration of the contrast media and will therefore not be allowed to participate in this research project.

b. Procedures in the event of adverse reaction. For this study, GFR measurements are performed in GCRCs located in hospitals with access to medications for medical emergencies, including medications listed in Appendix A, Table 3. In case of an adverse reaction, a physician should be called to evaluate the patient, including organ system involvement and severity.

Compensation

Participants will receive \$100 payment for their participation.

Ancillary study – Neuroimaging

For this *ancillary study*, an equal sample of 250 African Americans and 250 whites, aged 30-64 years matched for age, sex, and SES will be recruited from an expected 3,000 HANDLS Wave 3 participants. Based on the HANDLS Wave 2 follow-up agreement rates, it is estimated that approximately 1,000-1,500 of the 3,000 returning participants will need to be approached to enroll 500 eligible subjects into this ancillary study. The ancillary study will proceed concurrently with HANDLS Wave 3 and we expect this will provide ample time for recruitment. The exclusion criteria for this sub-study are more stringent than the criteria used for the parent study. These additional criteria enhance the scientific integrity of this neuroimaging sub-study and include additional safeguards that reduce risk to subjects by excluding subjects with contraindications to the MRI scans. Accordingly, additional exclusion criteria for HANDLS scan are: history of dementia, stroke or transient ischemic attack (TIA); history of carotid endarterectomy; contraindications to MRI scan (e.g., claustrophobia, indwelling ferromagnetic material); diagnosis of a terminal illness (e.g., metastatic cancer, end-stage liver or pulmonary diseases); within 6 months of active treatment of cancer (e.g., chemotherapy, biologic, radiation); or other neurological disorder (e.g., multiple sclerosis, Parkinson's disease).

If deemed potentially eligible to participate based on prior HANDLS data (which will be obtained from the NIA prior to scheduled visits), HANDLS investigators or a HANDLS SCAN investigator or coordinator will recruit participants during their MRV visit. The study will be described to participants, and they will be assured that they can decline participation in this ancillary study without affecting their status in the main protocol. For those agreeing to participate in this ancillary study, research subjects will sign an additional NIA HANDLS SCAN sub-study informed consent

form. After a brief screening interview for further determination of eligibility, participants will be scheduled for a visit to the University of Maryland School of Medicine. Transportation will be reimbursed. Written informed consent and HIPAA consent will be obtained at University of Maryland using documents approved by the NIA Institutional Review Board and the University of Maryland Human Research Protection Office. IRB approval for the studies will be obtained at the 5 participating institutions (National Institute on Aging, University of Maryland Baltimore County, University of Maryland School of Medicine, University of Pennsylvania, and George Mason University).

Methods

After obtaining written informed consent and HIPAA consent, participants will first be seen by Dr. Katzel or Dr. Seliger at the University of Maryland School of Medicine for a brief medical evaluation. The focus of this medical evaluation is to determine if there were any acute medical problems since their last HANDLS visit, and to review current medications, administer the MRI eligibility checklist, and assess whether there are any contraindications to the performing the supplemental testing. Participants will also undergo brief testing to supplement the HANDLS neurocognitive (executive function) and physical function batteries. To limit the additional testing burden, these proposed measures have been kept to a minimum. Lastly, they will undergo MR imaging. Participants will receive \$50 for HANDLS Scan.

Measures

Measure or Procedure	Estimated Timing	Location
MRI	1 hour	UMB
Cognitive tests	20 minutes	UMB
Corridor walk	10 minutes	UMB

Magnetic Resonance Imaging. Cranial MRI will be performed utilizing a Siemens Tim-Trio 3.0 Tesla unit in the Department of Diagnostic Radiology at the University of Maryland Medical Center. A sagittal scan of the brain will be obtained with T1 weighting (TR/TE/TI = 2660/12/1150), 5 mm slice thickness, 0.2 distance factor, 240 mm read FOV, 100% phase FOV, 1 signal average resulting in 24 slices. An axial scan will be obtained with FLAIR weighting (TR/TE/TI = 8000/67/2500), 5 mm slice thickness, 0.2 distance factor, 220 mm read FOV, 81.3% phase FOV, and 1 average for 26 slices. An axial scan of the brain will also be obtained with T1 weighting (TR/TE/TI = 2660/12/1150), 5 mm slice thickness, 0.2 distance factor, 220 mm read FOV, 100% phase FOV, and 1 average for 26 slices. An axial scan will be obtained with T2 weighting (TR/TE = 6000/103), 5 mm slice thickness, 0.2 distance factor, 220 mm read FOV, 100% phase FOV, and 2 averages for 26 slices. Volumetric T1-weighted magnetization prepared - rapid acquisition gradient echo (MP-RAGE) scans (TR/TE/TI/flip angle = 1900/2.4/900/9°) will be obtained with 256 mm read FOV, 100% phase FOV, and 1 average for 192 slices.

Diffusion tensor imaging (DTI) sequences will sample the diffusion tensor with a single-shot echo-planar spin echo imaging sequence in 128 non-collinear directions using diffusion-weighting gradients at a 'b' value of 1000 s/mm², and a non-diffusion weighted (B0) scan. Base sequence parameters of (TR/TE = 9300/84) will be applied in each of these directions with 2 mm slice thickness, 230 mm read FOV, 100% phase FOV, and 1 average for 68 slices. The DTI FOV will be

encompass the entire brain including the cerebellum, and provide isotropic resolution (2x2x2 mm³). The total scan time for the conventional, DTI, and T1-weighted volumetric sequences will be approximately 40 minutes.

Grooved Pegboard. The Grooved Pegboard Test requires manual dexterity, eye-hand coordination, and motor speed.⁷⁶ Participants are asked to insert 25 pegs (with a key on each end), as quickly as possible, into rows of slotted holes angled in different directions on a 4 × 4 inch pegboard. The pegs are rotated and inserted into the holes as quickly as possible, first with the dominant hand and then the non-dominant hand. Time to completion is scored for the performance of each hand. This test has proven particularly sensitive to cardiovascular risk factors and disease and chronic kidney disease in our investigations.

Contingency Naming Test. The Contingency Naming Test⁷⁷ was modeled after the Stroop Color-Word Test. It measures the ability to inhibit and switch mental set, functions thought to be mediated by a frontal-striatal network. It uses color and shape stimuli and, unlike the Stroop and other similar tests, does not make any demands on literacy or word knowledge. Part 1 requires the naming of colors; Part 2 requires the naming of shapes; and Part 3 requires the naming of color if outside and inside shapes match or the naming of the primary shape if the outside and inside shapes don't match.

Timed Walk. Self-selected walking speed will be measured during the course of a 10-meter walk. A reduced self-selected walking velocity, a commonly measured index of function performance has been shown to predict subsequent morbidity and mortality, and correlates with severity of white matter disease.⁷⁸

Post-acquisition data processing

MRI Image Analyses. Atlas warping and labeling using HAMMER: Manually labeling a large number of regions of interest (ROIs), in order to obtain a complete picture of brain atrophy is prohibitively costly in terms of time and effort, and less likely to be feasible in the typical clinical setting. As a first step toward obtaining a more comprehensive set of volumetric measurements from the entire brain, Dr. Davatzikos and colleagues previously developed and used in a variety of studies an atlas-based labeling and parcellation method.^{79,80} Atlas-based methods start with a digital atlas, which has been partitioned to a number of ROIs. In our case we use an atlas previously labeled at the Montreal Neurological Institute into a set of 92 ROIs that includes all major cortical gyri and subcortical structures, as well as lobar white matter subdivisions. In order to define these ROIs on individual scans, we use a 3D elastic warping computer algorithm, which finds the spatial transformation (warping) of the template that best matches the morphology of a new scan. The labels of the atlas are then transferred to the individual scan via this spatial transformation. Volumes of these ROIs are readily determined by summing up all voxels within an ROI. Lesion load within each ROI is also determined after initial segmentation of the lesions, as described later in the proposal. This way, we obtain a set of 92 volumes for each brain scan in our studies, which is subsequently treated in multivariate statistical analysis frameworks seeking group differences and associations between these regional brain volumes and clinical, neuropsychological, or demographic variables.^{81,82}

Diffusion Tensor Imaging Analyses. As a first step, we will spatially normalize the DTI data to a template (as explained in preliminary results). The statistical analysis will consist of (1) voxel-wise group based analysis of FA and ADC computed from the tensors; (2) regression of these scalar maps with respect to age and clinical scores; (3) ROI-based correlation with age and other relevant

variables with ROIs chosen on an atlas; and, (4) tract-based analysis across groups and their correlation with age and clinical variables.

Sample size

We plan to perform a number of different analyses, the most restrictive of which examines a structural equation model. Sample size determination for structural equation modeling is considerably more complex than for bivariate relations or regression models. At one extreme, Raykov and Marcoulides⁸³ suggest that “a cautious and simplified attempt at a rule of thumb might suggest that sample size would desirably be more than 10 times the number of free model parameters.” Sartorra and Sarris⁸⁴ describe an elegant method of sample size computation that requires specification of all parameters in null and alternative models. This is impossible at the beginning of the study for the complex models we are hypothesizing. Even the standard textbook by Bollen (p. 346)⁸⁵ states that such an approach of specifying the parameters “is somewhat arbitrary”. The parameters may be estimable to some degree after some preliminary data on the correlations among observed variables becomes available. A series of 3 SAS program written by Co-Investigator Rosenberger can be used to determine appropriate power and sample size considerations given reasonable estimations of all the parameters. As we obtain more data during the course of the study, we will use Sartorra and Sarris’s method to determine if the sample size goals are adequate.

We need another approach to determine the sample size for testing our primary hypotheses, which is provided by MacCallum and colleagues.⁸⁶ The Table below shows the sample size required to determine an appropriate level of goodness of fit for our most complex model. We have assumed correlated error structures where appropriate. Here we give the power of the test for an exact fitting model, and the effect size represents the model discrepancy in the population, as measured by Steiger’s deviation of the root mean square error of approximation (RMSEA).⁸⁷ Brown and Cudeck⁸⁸ use empirical evidence to suggest that the RMSEA effect size should be less than 0.05 for a good-fitting model. Based on this preliminary analysis, it would appear that the proposed 500 subjects would be adequate for testing goodness of fit at an effect size of between 0.015 and 0.020, which is well below Brown and Cudeck’s recommended threshold.

Table. Minimum sample size for 90% power for testing goodness of fit of structural equations model. Effect size represents deviation from an exact fit.⁸⁶

Effect size	Minimum Sample Size
0.010	1238
0.015	550
0.020	310
0.025	200

Risks and Benefits

Risks. There are potential risks associated with some of the procedures included in this study. However, the procedures have been planned by the investigators to minimize the danger of any

major complication. All medical procedures will be supervised by qualified medical personnel who will carefully monitor the participant.

Neuropsychological Testing. Although there are no significant risks to subjects during psychological and neuropsychological testing, every effort will be made to make participants feel comfortable. The only risk of this study is “psychologic discomfort.” HANDLS participants are accustomed to these procedures because we administered neuropsychological tests as part of their baseline examination.

Physical Function Tests. These tests involve a timed walk. No tests will be performed before a physician examines participants. A crash cart and emergency medications are available in the area where these tests are performed.

Magnetic Resonance Imaging (MR). Medical personnel associated with this sub-study will carefully screen participants for MRI contraindications (e.g., pacemaker, aneurysm clip, metallic prostheses). MRI will not be performed if we find a contraindication. If there is a history of occupational exposure to metallic fragments or questionable history of other exposures to metal, pilot x-rays are performed of the skull and orbits to evaluate the presence of metal. Tests will not be performed if there are contraindications.

Medical personnel associated with this sub-study will interview participants about claustrophobia before the procedure. We will stop the MRI if the participant experiences any discomfort or anxiety. All MRI scans will be reviewed for unexpected abnormalities (e.g., mass lesion, blood) by a licensed neuroradiologist at the University of Maryland School of Medicine. We will refer participants with abnormalities requiring further follow-up to their physician for further assessment. If they do not have a physician then we will refer them to facilities available in Baltimore City as part of the HANDLS referral network. Study co-investigators Drs. Katzel and Seliger are available on site to deal with any medical issues, adverse events, unanticipated problems to participants or others that arise during the study. We will include twenty-four hour contact information on the consent forms.

Benefits. Participants may benefit from the neuroimaging procedures by acquiring a "baseline" scan that can be used for comparison purposes at a later date. This may a pertinent benefit as patients with cardiovascular risk factors are at increased risk for cerebral infarction. In addition, the brain scans may find clinically significant results that warrant follow-up outside of this study.

We believe that the benefits associated with this study will exceed the risks, thereby resulting in a low risk:benefit ratio for participants.

Compensation

Participants will receive \$50 payment for their participation and transportation reimbursement.

Publications based on the data include:

Merritt MM, Abernethy DR, Evans MK, Sollers JJ III, Zonderman AB, and Thayer JT: Cardiovascular Reactivity to perception of affect among older African-American adults. *Psychophysiology*, 39 (Supplement 1): p. S9, October 2002.

Evans MK, Zonderman AB, and Johnson WR: Letter to the editor regarding the Ridker article of November 14. *The New England Journal of Medicine*. January 2003

Kitner-Triolo MH, Donohue JE, Evans MK, and Zonderman AB: Association of age with attention and executive function mediated by SES. Poster presented at the International Neuropsychological Society Conference. Honolulu, Hawaii. February 2003.

Evans MK, Zonderman AB, and Johnson WR. C-reactive protein in the prediction of cardiovascular events. *N Engl J Med*. Mar 13 2003; 348(11): 1059-1061; author reply 1059-1061.

Merritt MM, Sollers JJ III, Evans MK, Zonderman AB, and Thayer J T: Relationships among spectral measures of baroreflex sensitivity and indices of cardiac vagal control. *Biomedical Sciences Instrumentation*, April 2003, 39: p. 193-198.

Thayer JF, Merritt MM, Sollers JJ, 3rd, et al. Effect of angiotensin-converting enzyme insertion/deletion polymorphism DD genotype on high-frequency heart rate variability in African Americans. *Am J Cardiol*. Dec 15 2003;92(12):1487-1490.

Terracciano A, Merritt MM, Zonderman AB, Evans MK. Personality traits and sex differences in emotion recognition among African-Americans and Caucasians. *Annals New York Academy Sciences* 2003;1000:309-312.

Mager, D. E., Merritt, M. M., Kasturi, J., Witkin, L. R., Urdiqui-MacDonald, M., Sollers, J. J. III, Evans, M. K., Zonderman, A. B., Abernethy, D. R. & Thayer, J. T. (2004, March). Kullback-Leibler clustering of continuous wavelet transform measures of heart rate variability. *Biomedical Sciences Instrumentation*, 40: 337-342.

Nguyen HT, Kitner-Triolo M, Evans MK, Zonderman AB. Factorial invariance of the CES-D in low socioeconomic status African Americans compared with a nationally representative sample. *Psychiatry Research*, 2004;126(2):177-87.

Sollers JJ, Merritt MM, Silver RA, Evans MK, Zonderman AB, Thayer JF. Understanding blood pressure variability: Spectral indices as a function of gender and age. *Biomedical Sciences Instrumentation*. 2005;41:43-47

Nguyen HT, Evans MK, Zonderman AB. Influence of medical conditions on executive and memory functions in low socioeconomic status African Americans. *Arch Clin Neuropsychol*. 2007 Aug;22(6):689-98

Dotson VM, Kitner-Triolo M, Evans MK, Zonderman AB. Literacy-based normative data for low socioeconomic status African Americans. *The Clinical Neuropsychologist*. 2008;epub:1 - 29.

Study Population – Gender and Ethnic Inclusion

In this study we have collected a representative sample of Baltimore City residents.

Plan to re-contact participants for wave 3. The HANDLS study has recruited a representative sample of 3724 whites and African Americans between 30 and 64 years old from 12 census tracts in Baltimore City in both low and high socioeconomic strata as a fixed cohort following the overall design. We have used several methods to remain in contact with our participants since they initially enrolled in HANDLS. Specific examples include sending regular mailings such as newsletters, holiday and birthday cards to the addresses we have on file, participation in the wave 2 interim study, mailing study updates and reminders with change of address cards, and periodic reviews of Baltimore City judicial system public records and the National Death Index database. While this does

allow us to remain in contact with many of our participants, there still exists a sub-set of participants for whom traditional methods will not be successful.

For Wave 3 we plan to hire a tracing and tracking specialist whose primary responsibility will be to focus on conducting investigative fieldwork and extensive tracing & tracking procedures to locate missing participants. This will require (a) physically driving through all identified HANDLS study neighborhoods in Baltimore City to previously known addresses for missing participants, communicating with current residents (and or neighbors) of identified households to assist in locating participants; (b) contacting participant's family or friends identified by the participant as persons to be reached if participant cannot be located (c) using search engines on the internet, Baltimore City judicial system public records, National Death Index, Division of Vital Records, and similar methods to locate current residence or to verify status of missing participants; and, (d) other tracing and tracking methods developed over time and with experience.

Including this strategy will allow us to make every possible effort to locate as many of our participants as possible. It is particularly crucial as we move into the first follow-up re-examination phase of the study.

Vertebrate Animals. None.

Consultants and Collaborating Agencies. Some staff positions in this study are filled through contract with MedStar Research Institute. The Principal Investigators will provide technical supervision. We also have an intra-agency agreement with the United States Department of Agriculture (USDA) to provide consultation on the nutritional domain of the study. Individual consultants are listed at the beginning of the protocol with institutional affiliations.

Curriculum Vitae and Biosketches. See attachments

Compliance. This trial will be conducted in compliance with this submitted protocol, U.S. Department of Health and Human Services, National Institutes of Health, Food and Drug Administration, ICH, and all applicable, state and local requirements.

Consent Process

There are three phases to the study. The first phase occurs in the field, at the medical research vehicles (MRVs). Prior to coming to the medical research vehicles for their study visit, participants will receive a HANDLS participant invitation packet that will include a letter inviting them to participate in the next study visit, an informed consent booklet, all consent forms for this wave of the study, and a meet the staff identification sheet identifying the role of each of the HANDLS staff members. They will be asked to call the study visit scheduler to make their appointment for the MRV visit. Participants will be instructed to read all consent documents carefully prior to calling for their appointment. The study visit scheduler will verify that participants have received and read copies of all documents. She may suggest participants review or discuss their participation with their families and or primary care physician, if they so choose.

Among the preparations for their examinations on the medical research vehicles, participants are asked to bring their copies of unsigned consent documents to their appointments (if they fail to bring their copies, additional copies are provided on the MRV). Before they sign their consent documents, participants view a consent film about the HANDLS study that explains the purpose of the study and all procedures they have previously reviewed in the informed consents. The HANDLS staff reviews the consent documents with participants a final time, ensures the participant has a clear understanding

of the study, the degree of risk, potential benefits, and alternatives and provides the participant with an opportunity to ask questions and consider their decision to participate in this next wave of the HANDLS study.

If participants agree to take part, signatures will be obtained using an IRB approved hard-copy of the informed consent document or electronically using a PC tablet. The informed consent document is given to the participant for final review and is then asked to sign the consent forms. Regardless of how signatures are obtained, HANDLS staff provides participants with printed copies for their records and a copy is placed in the research medical record. HANDLS staff sends participants copies of all signed informed consent documents with the results from their examinations.

Phase 2 Consent Procedures

This consent will either be done in person and obtained signatures or as an oral consent. When conducted as an oral consent, it will be read to the informant. All elements required by 45 CFR 46.116 is included, as well as required documentation of the oral consenting process.

As part of the MRV visit (phase 1) participants will be invited to take part in phase 2 of HANDLS wave 3, a telephone interview. A copy of the consent for phase 2 will be given to and reviewed with the participant before they complete their MRV visit. They will be invited to participate in phase 2, as a telephone interview. Before they sign the consent document for phase 2, all study procedures will be explained to the participant. They will then be invited to sign the consent for phase 2. Participants will be notified that an interviewer will be contacting them shortly after their MRV visit. The telephone interviewer will contact the participant (by phone or mail) and determined the candidates are still interested in participating in phase 2 of the study. Prior to administering the questionnaires, the interviewer confirms that they have a printed copy of the informed consent document for phase 2 of the study. The interviewer reads the informed consent document verbatim and requests the participant reads along with them. The interviewer reviews the study procedures with candidates, and confirms that the candidates have clear understandings of the study, the degree of risk, potential benefits, and alternatives. Candidates may ask questions and/or further review the informed consent document in the privacy of their homes and at their convenience. The telephone interviewer may suggest that candidates review or discuss their participation with their families, friends and/or health care providers. If requested, the field interviewer will call back at a later date to complete the enrollment. Alternately, candidates may choose complete the consent process by answering the oral documentation questions below. Once the questions have been answered, a telephone witness will certify the participant has agreed by signing the oral documentation portion of the consent form.

ORAL DOCUMENTATION

Is there anything you would like me to repeat? (Responded) ____ Yes ____ No

Have you understood everything I have told you? (Responded) ____ Yes ____ No

Do you have any questions? (Responded) ____ Yes ____ No

Do you agree to participate? (Responded) ____ Yes ____ No

I have read the above informed consent over the phone to (print name of person being consented) _____ and s/he has agreed to answer the questions and participate in this research study. **Signature recorded on last page*

Print name of person reading this consent_____

Print name of witness who observed: _____

Date_____ Time: _____

Appendices

Informed Consent Document for HANDLS Wave 3 MRV Visit– Phase I

Informed Consent Document for HIV – Wave 3

Informed Consent Document for Genetics Testing – Wave 3

Informed Consent Document for HANDLS Wave 3 Telephone Interview– Phase II

HIPAA Document for HANDLS Wave 3

Informed Consent Document for GFR study – HANDLS Wave 3

Informed Consent Document for DTI study – HANDLS Wave 3

Informed Consent Document for HANDLS Wave 2 – “The Association of Personality and Socioeconomic Status with Health Status - An Interim Follow-up Study”

Forms for GFR Protocol

Tables of Adverse Reactions to Iodinated Radiographic Contrast Material during Imaging Procedures

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